Synopsis

Name of Research Scholar: Vivek Kumar

Registration number of Research Scholar: 23/Ph.D./DPSRU/2020

Name of Supervisors: Dr. D.P. Pathak and Dr. Nidhi Sandal

Title of Thesis: Synthesis and Characterisation of Prussian blue insoluble for decorporation of Cesium and Thallium

1. Introduction:

The expansion of the nuclear production has generated numerous effluents comprising radionuclides like Cs, Sr, Tl, Ba, Co etc. released from medical, military or commercial practices. Cesium has half-life of 30 years and it mimic potassium once enters the body. Thus, it is biological one of the most significant radionuclides. On account of their extensive exposure, these radioactive materials enter the body through ingestion, inhalation or skin contact and have the greatest potential to cause harmful effects to the human and animals. The uses of Prussian blue for decorporation of cesium and thallium initiated in 1960s. Prussian blue is described as a deep blue pigment that is produced when the oxidation of ferrous ferrocyanide salts occurs. It contains ferric hexacyanoferrate (II) in a cubic lattice crystal structure. It is insoluble in water but also tends to form a colloid thus can exist in either colloidal or water-soluble form, and an insoluble form. It was recommended for thallium toxicity in 1970s and still in use. After the incident of Goiania in Brazil, Prussian blue insoluble was recommended for decorporation of a large-scale radiation calamity. In October 2003, USFDA approved Radiogardase®-Cs, 500 mg hard capsule containing Ferric(III) hexacyanoferrate(II) (Prussian blue insoluble), intended for treatment of individuals who are exposed to radioactive cesium or thallium. Several studies has been evaluated the purpose of Prussian blue as decorporation agent for enhancement of excretion of radioactive or nonradioactive isotopes of radionuclides. There are few in vitro published studies available on binding efficiency of insoluble PB correlatively with chemical and physical circumstances such as temperature, storage conditions, pH, moisture content and particle size.

The pigment Prussian blue contains iron cations, water molecules and cyanide anions. PB pigments, generally either the "soluble" KFe^{III} $[Fe^{II}$ $(CN)_6]_3.xH_2O$ or the "insoluble"

$Fe_4^{III}[Fe^{II}(CN)_6]_3$. xH_2O forms are present. PB is a chemical moiety of crystal lattice of ferric hexacyanoferrate $Fe_4^{III}[Fe^{II}(CN)_6]_3$. There are typically 14-16 water molecules coordinated to the Prussian blue molecule. The crucial metal binding mechanism for Prussian blue is monovalent cesium ion exchange with the hydrogen ion (H^+) or from water bound hydronium ion (H_3O^+) in Prussian blue crystal lattice. The agent like Prussian blue Insoluble, which does not absorbed through gut and can be considered as effective and safe for internal decontamination of radioactive or non-radioactive thallium or cesium. It is important to note that the synthesis process may also influence the oxidation state of iron (Fe (II) vs. Fe (III)) in the ion exchange; change in precursor leads to altered arrangement in the crystalline structure, the vacancy of Ferro/ferricyanide positions, the size of ion exchange cage; the possible incorporation of water molecules. In turn water molecules may affect the coordination sphere and cyanide group reactivity. Faustino compares the physicochemical characteristics of three API batches which were tested under various gastrointestinal (GI) pH conditions and transit times based on physiologically relevant conditions. In addition, several in vitro and animal studies used a variety of hexacyanoferrate ensuing from various precursors, synthesis method and manufacturing drying circumstances known to affect the binding efficiency.

2. Rationale of Research work:

PB does not absorbed through gut and is as effective and safe for internal decontamination of radioactive or non-radioactive thallium or cesium. It is important to mention here that even though it's an essential radionuclide decorporation agent, but no country has included its monograph in any official pharmacopoeias. The literature has insufficient information on in-vitro studies and in-vivo efficacy of cesium and thallium to Prussian blue under various physiochemical circumstances. Prussian blue as an API is not available in India and its procurement from outside is difficult and time consuming. Details to understand the chemistry used for investigation of Prussian has never been discussed. Over to these, no available data of developed and validated method for synthesis is there and Preparation methods vary widely. Spectral and physical properties Vary with the method of synthesis used. The efficacy of Prussian blue available commercially depends significantly on the process of synthesis. Also, Prussian blue available in market is usually heavily contaminated with various impurities like salts of some metals and ions which are formed as side products from the reaction, which significantly affect cesium binding efficacy. Additionally, this

study will evaluate the effect of such factors as particle size and storage conditions on the cesium binding capacity of synthesized PB, commercially available active pharmaceutical ingredients (APIs) and Radiogardase®-Cs. Several *in vitro* and animal studies used a variety of hexacyanoferrate ensuing from various precursors, synthesis method and manufacturing drying circumstances known to affect the binding efficiency. Thus, there is urgent need for development of standard method of synthesis, optimization of synthesis parameters involved in synthesis. As the commercially available APIs of PB insoluble are not intended for treatment of Cesium or thallium poisoning so, impurity profiling is required. Thus, characterization and method validation of synthesized PB insoluble is necessary. Therefore, the present study was focused on chemical as well as physical characteristics of Prussian blue insoluble. Prussian blue insoluble will be synthesized by direct and indirect methods and estimation of various physical and chemical parameters such as particle size, pH of medium, moisture content and temperature. Hence, the present study will comprise of synthesize and characterize various physical and chemical parameters of Prussian blue insoluble and compared with commercially available Prussian blue APIs and Radiogardase®-Cs.

Objectives: The objective of this study is to synthesize and characterize various physical and chemical parameters of Prussian blue insoluble and compared with commercially available Prussian blue APIs and Radiogardase®-Cs.

The specific objectives aimed in the present work are as follows:

- To synthesise Ferric (III) hexacyanoferrate (II) i.e. Prussian blue insoluble
- To Characterize Ferric (III) hexacyanoferrate (II) and its comparison with commercially available Prussian blue (PB) APIs and Radiogardase®-Cs
- Evaluation of *in-vivo* removal efficacy of synthesized PB insoluble for nonradioactive isotopes of Cs and thallium in animal model and its toxicity studies.

3. Plan of work and Methodology:

3.1 Synthesis of Ferric (III) hexacyanoferrate (II) i.e. Prussian blue

Direct method

The direct method for synthesizing ferric hexacyanoferrate (II) involves one-step reaction between potassium ferrocyanide and ferric chloride. Solutions of 40mM ferric chloride and potassium

ferrocyanide were prepared in water, separately. After preparation of both solutions, add potassium ferrocyanide solution over ferric chloride solution to yield Ferric hexacyanoferrate (II). The resulting mixture was constantly stirred during this process. The product was centrifuged, and water was decanted. The residue obtained was washed 5 times with Millipore water.

Indirect Method

The indirect method for synthesis of Ferric hexacyanoferrate (II) involved two-step reaction. Solution of 0.3 M ferrous sulphate and 0.1 M potassium ferrocyanide was prepared, separately. After this, the solution of potassium ferrocyanide was poured in the solution of ferrous sulphate in equal volumes. It resulted in the formation of Berlin white, and kept for 2h at 60 °C at atmospheric conditions. After aging, Berlin white was oxidized to Prussian blue (Ferric hexacyanoferrate (II)) with the help of Hydrogen peroxide and washed with warm water and desiccated in a tray dryer at 80 °C for 2 h.

3.2 To Characterize Ferric (III) hexacyanoferrate (II) and its comparison with commercially available Prussian blue (PB) APIs and Radiogardase®-Cs

The characterization of Ferric (III) hexacyanoferrate (II) and its comparison with commercially available Prussian blue (PB) APIs and Radiogardase®-Cs will be characterized using various instruments and techniques. (Limit tests, Iron content, FTIR, UV/Vis Spectrophotometer, SEM, TGA, XPS, LDS analyser, Binding efficacy, AAS etc.)

- **Maximum binding capacity**

Estimation of in-vitro binding efficiency of Synthesized Prussian blue

Adsorption isotherm model

The maximum thallium adsorption capacity of PB (mg g^{-1}) will be determined by subjecting the data to Langmuir and Freundlich adsorption isotherm models. The Langmuir adsorption isotherm model assumes monolayer adsorption onto a surface containing a finite number of adsorption sites. The linear equation of Langmuir adsorption isotherm is represented by:

$$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m}$$

Where C_e (mg L^{-1}) is concentration of thallium at equilibrium, q_e (mg g^{-1}) amount of thallium adsorbed at equilibrium, q_m (mg g^{-1}) maximum capacity of adsorbent and K_L (L mg^{-1}) constant

related to the energy of adsorption. Line Plotted between C_e/q_e *vs* C_e and from which the constants q_m and K_L calculated

The Freundlich adsorption isotherm model suggests the relative distribution of the energy and the heterogeneity of the adsorbate sites. The linearised Freundlich adsorption isotherm is represented by:

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e$$

Where K_f (L mg^{-1}) is a Freundlich constant characteristic of a particular adsorption isotherm and n (dimensionless) is a Freundlich constant representing the adsorption intensity. A plot of ln q_e *vs* ln C_e would give a straight line from which constants K_f and n calculated.

3.3 Evaluation of *in-vivo* removal efficacy of synthesized PB insoluble for nonradioactive isotopes of Cs and thallium in animal model and its toxicity studies.

The *in-vivo* efficiency of PB will be assessed against the non-radioactive Cs and Tl. Experimental Animals will consist of Wistar rat (150-200g) of either sex will be used for the assessment in-vivo Cs and Tl removal efficiency of PB insoluble.PB synthesized will be suspended in Milli-Q water and dosed once a day, orally till experimental period.

The animals will be divided into different groups (n=6) and treated as following:

- ✓ Non-radioactive isotopes of Cs or Tl suspended in water.
- ✓ Treated with PB synthesized and Radiogardase® -Cs

The duration of experiment will be 14-28 days.

Collection and Analysis of Urine, Fecal and Organs: At the end of the experiment urine, fecal and organs like kidney, liver, spleen, heart, brain, stomach, intestine, colon, muscle and bone will be collected and digested with 5% Nitric acid. The samples will be homogenized, and supernatant will be analyzed for non-radioactive metal ions using AAS.

3.4 Toxicity studies: Acute and sub-acute oral toxicity study

The toxicity studies will be performed as per OECD guidelines.

4. Work done:

- Synthesise of Ferric (III) hexacyanoferrate (II) i.e. Prussian blue insoluble.
- Characterization of Ferric (III) hexacyanoferrate (II) and its comparison with commercially available Prussian blue (PB) APIs and Radiogardase®-Cs.

5. Expected outcome/ outcome achieved:

- Finalization of specifications of PB as Active pharmaceutical ingredient.
- Scale up batches.

6. Publications/workshops/conferences/patents:

Communicated research paper title **"Quantitative attributes of synthesized Ferric (III) hexacyanoferrate (II) and its comparison with commercially available insoluble Prussian blue"** in Journal of Biomedical Materials Research: Part B - Applied Biomaterials

Vivek Kumar **(Research scholar)** **Reg. No.: 23/PhD/DPSRU/2020**	**Dr. D.P. Pathak** **Signature of Supervisor**	**Dr. Nidhi Sandal** **Signature of Supervisor**